T06020 "S2S09250

FIG. 1A.

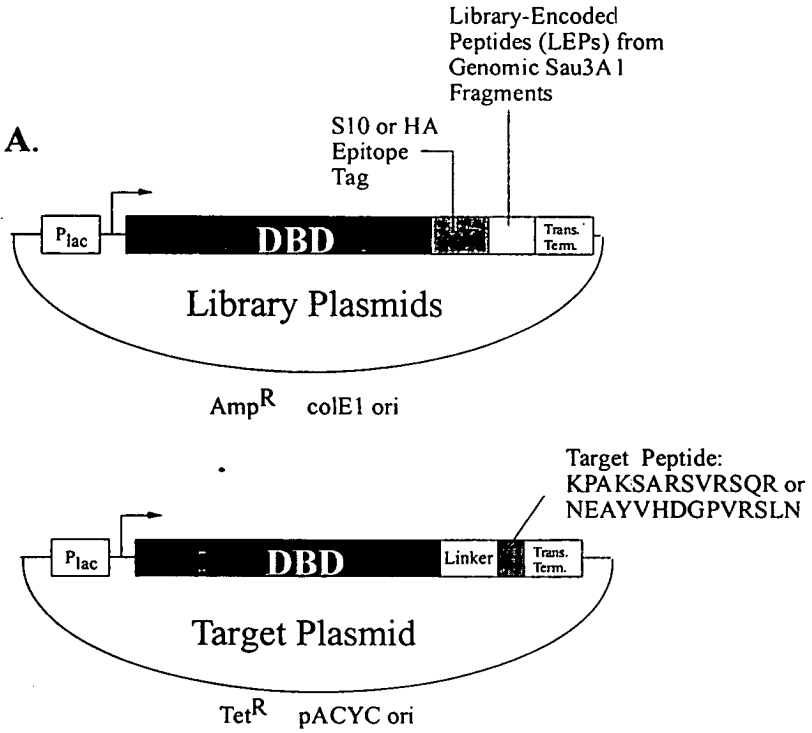

FIG. 1B.

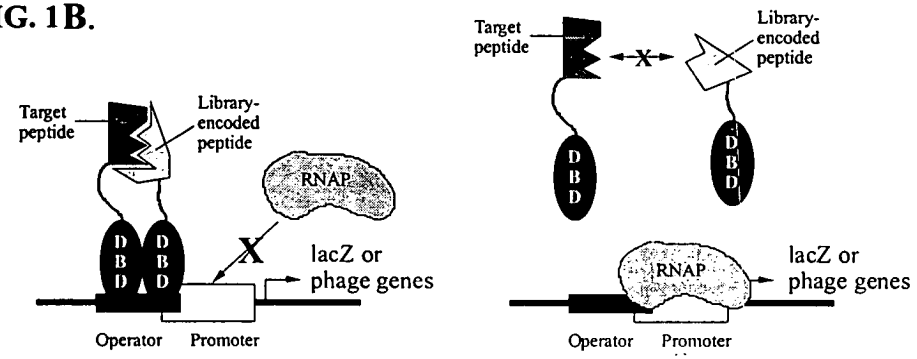

FIG. 2

13mer target: KPAKSARSVRSQR
27mer Library-encoded peptide:
THTTSQTTLRDPDVYAGARWVTWRVGA

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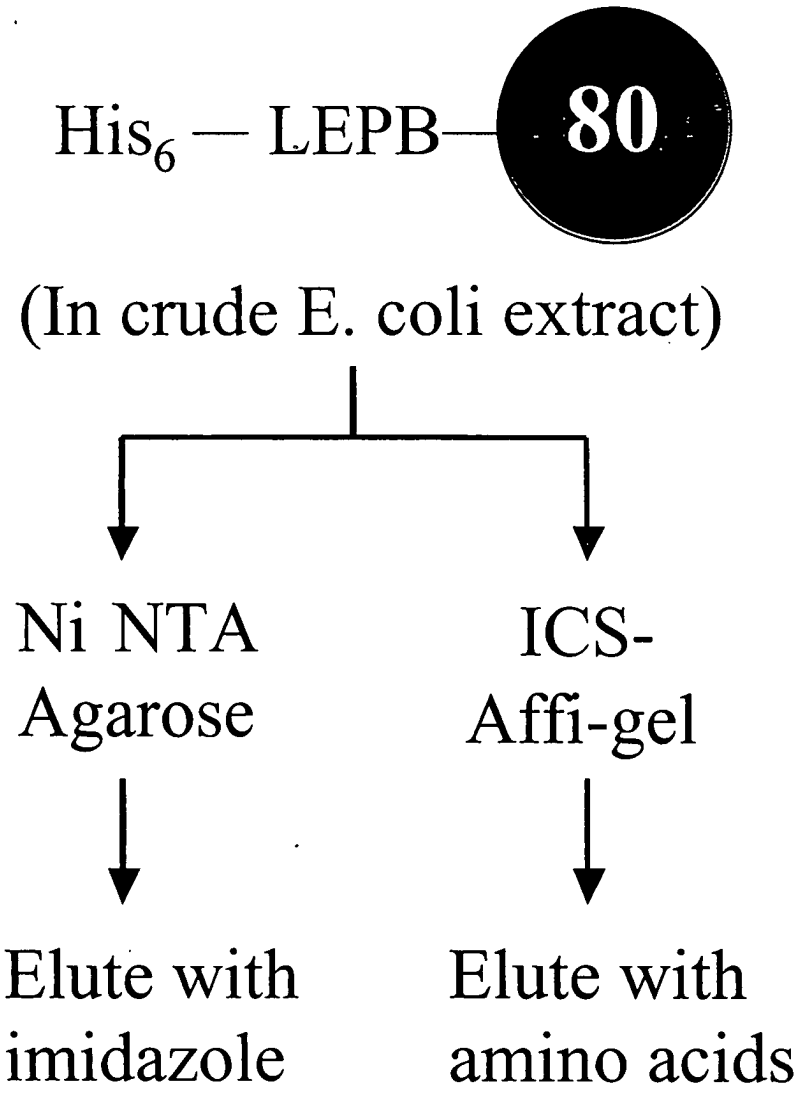
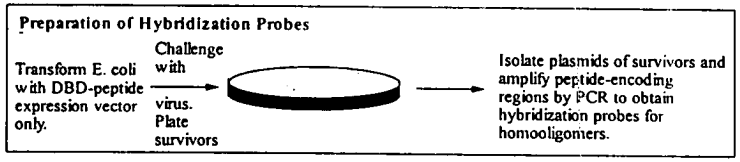


FIG. 3



Selection Protocol

Transform E. coli (JH372) with optimized DBD-target peptide and DBD-peptide library-encoding plasmids. Grow several generations.

Challenge with phage lambda

Phage-resistant cells with functional Repressor fusions. mixture of homooligomers and LEP-target peptide complexes.

Plate and replica plate. Probe permeabilized cells with radioactive probes corresponding to homooligomeric peptide-encoding DNAs

Pick colonies that do not light up for further analysis.

Isolate plasmids from individual colonies. Cure of target-encoding plasmid by cleavage with FseI and digestion of linear DNA with T4 DNA polymerase. Retransform library-encoding plasmid into fresh JH372 cells. Plate on X-gal plates.

Isolate DBD-LEP-encoding plasmid. Transform into fresh cells containing the target plasmid. Plate on X-gal indicator plates.

Discard. Represents homooligomer that slipped through the hybridization screen

Blue colonies

White colonies

Discard. Artifact due to promoter mutation in original target-encoding plasmid.

Analyze peptide-peptide complexes biochemically

FIG. 4

TOS020 "S2S08260

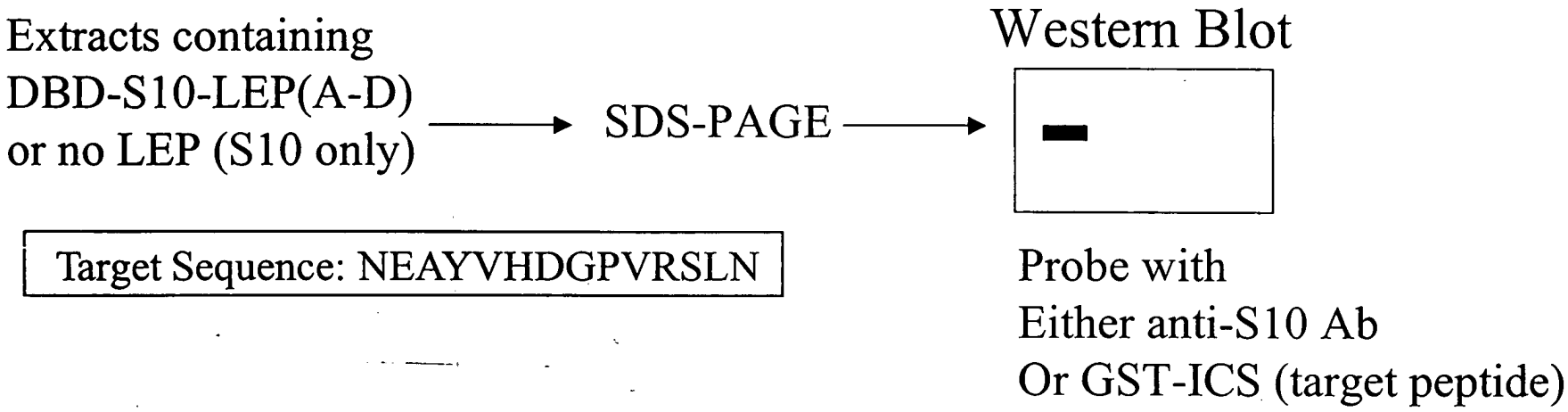
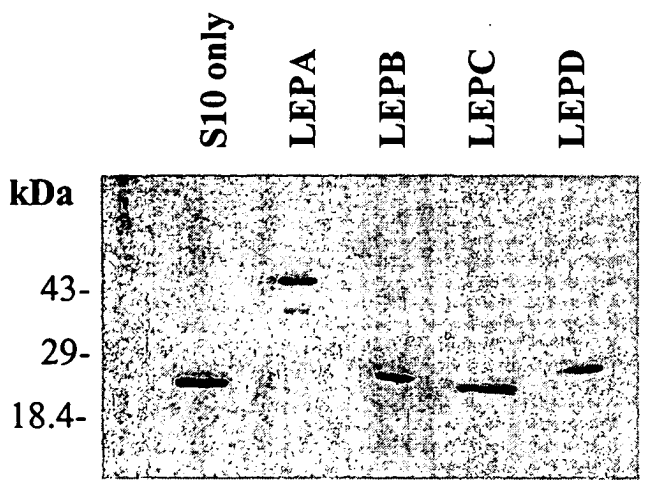
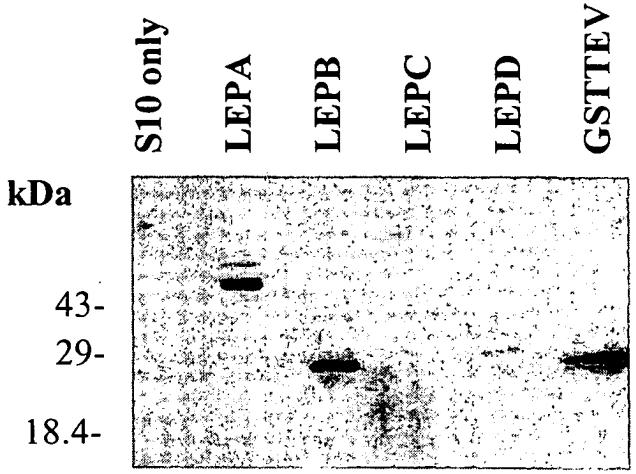


FIG. 5



Anti-S10 Ab



GST-ICS (target peptide)

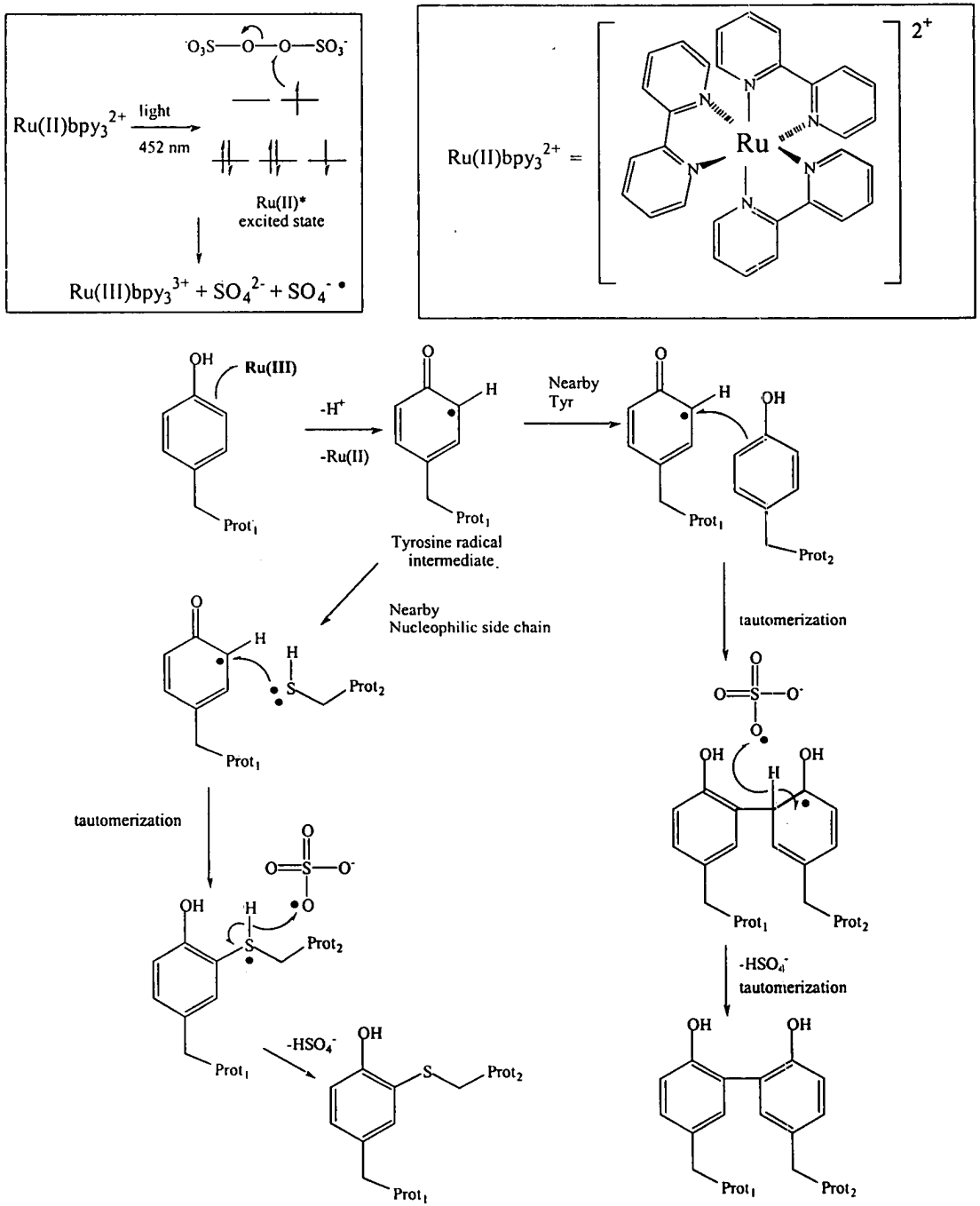

$O_3S—O—O—SO_3^-$
$Ru(II)bpy_3^{2+}$
light
452 nm
Ru(II)*
excited state
$Ru(III)bpy_3^{3+} + SO_4^{2-} + SO_4^{-\bullet}$
$Ru(II)bpy_3^{2+}$ =
Ru
2^+
Ru(III)
$-H^+$
-Ru(II)
Prot1
Tyrosine radical
intermediate
Nearby
Tyr
Prot2
Nearby
Nucleophilic side chain
tautomerization
$-HSO_4^-$

FIG. 6

FIG. 7

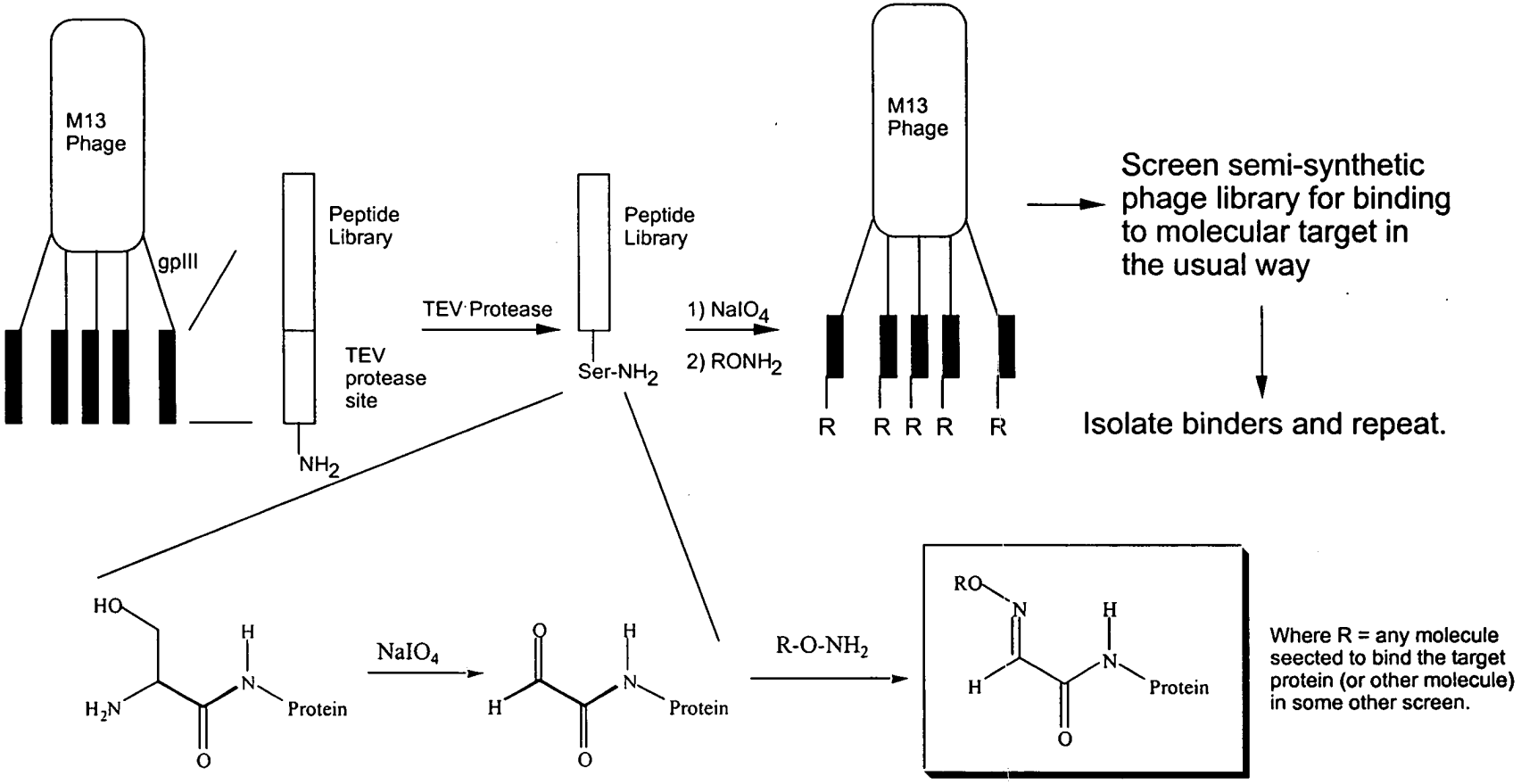

M13 Phage
gpIII
Peptide Library
TEV protease site
NH2
TEV Protease
Peptide Library
Ser-NH2
1) NaIO4
2) RONH2
M13 Phage
R R R R R
Screen semi-synthetic phage library for binding to molecular target in the usual way
Isolate binders and repeat.
HO
H2N
H
N
Protein
O
NaIO4
O
H
N
Protein
O
R-O-NH2
RO
N
H
H
N
Protein
O
Where R = any molecule seected to bind the target protein (or other molecule) in some other screen.

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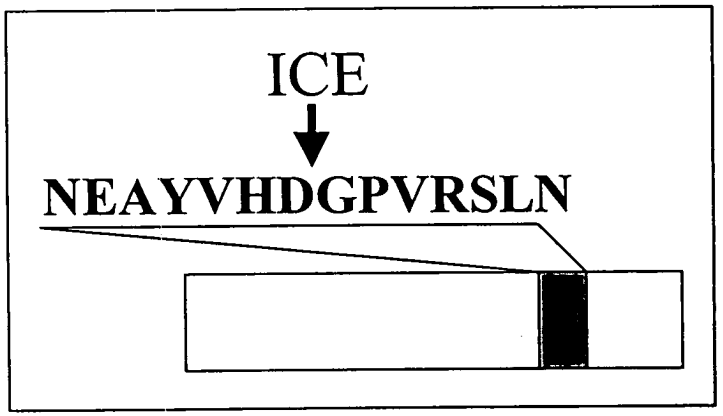
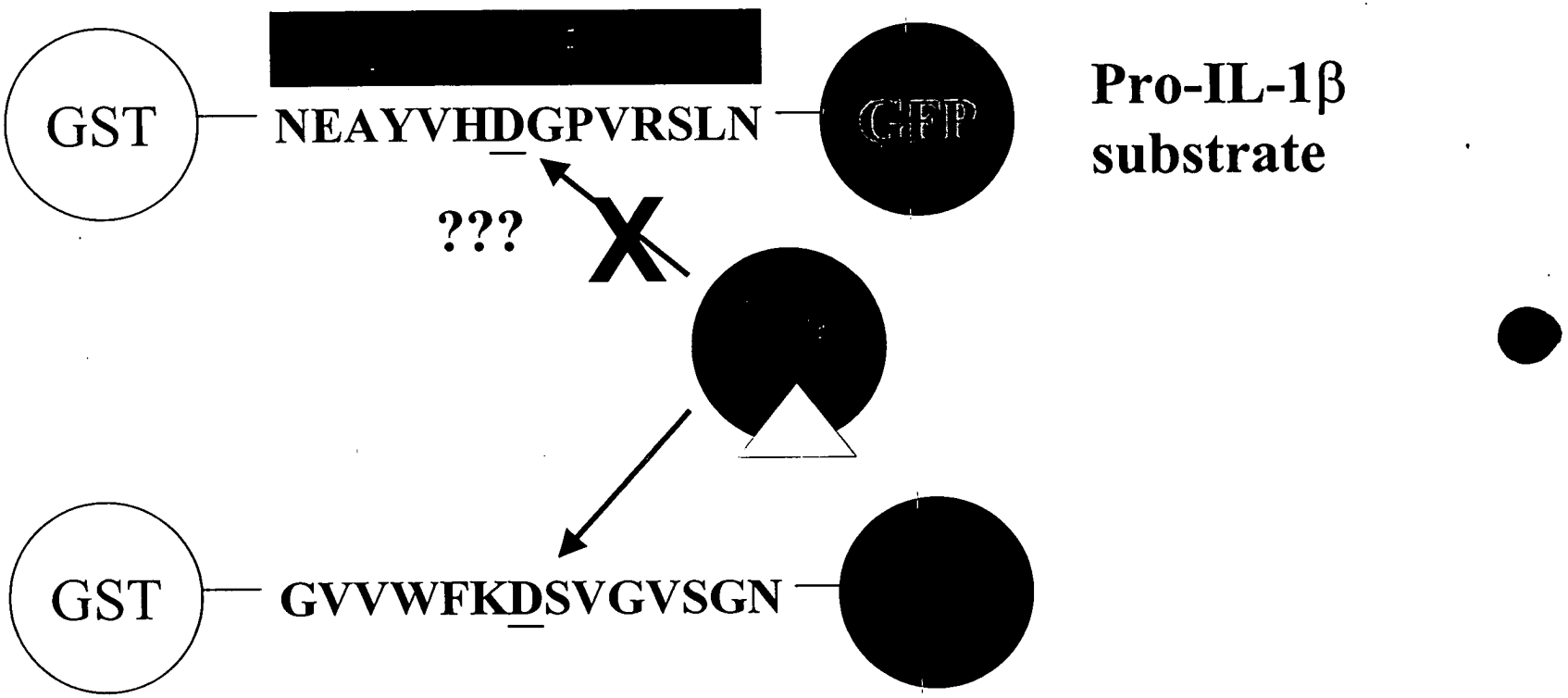
FIG. 8





T0602D "S2508250

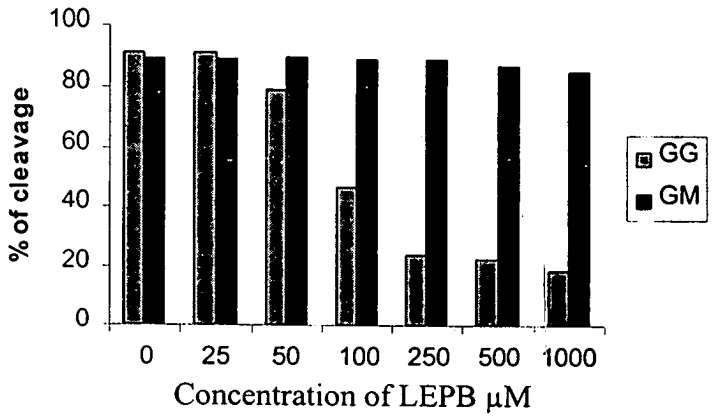


FIG. 9